Industrial Crops and Products 32 (2010) 1–6

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Analysis of breeding systems, ploidy, and the role of hexaploids in three *Hypericum perforatum* L. populations

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ARTICLE INFO

Article history:
Received 20 November 2009
Received in revised form 15 January 2010
Accepted 18 January 2010

Keywords:
St. John's wort
Flow cytometry
Ploidy
Hexaploid
Reproduction

ABSTRACT

Hexaploid seeds are produced by predominantly tetraploid populations of *Hypericum perforatum*, but the fate of hexaploid seedlings and their reproductive behavior have not been closely examined. We used flow cytometry to analyze single seeds and individual plant samples of three accessions of *H. perforatum* to determine ploidy levels and reproductive pathways. Seed samples of all three accessions were facultative apomicts, with tetraploid cytotype predominant (85–91%) and a lower frequency of hexaploids (9–14%), with diploids (5%) detected in only one population. Seedling populations consisted of tetraploids (87–97%) and hexaploids (3–13%). Hexaploid embryos are most likely generated by a 2*n* gamete of the tetraploid and fertilized by a normal, reduced tetraploid male gamete. These hexaploids are expected to produce unbalanced gametes because they possess chromosome complements that include two triploid sets originally derived from two different species. The observation that some tetraploid seeds had endosperm with high cellular DNA content indicates that some unbalanced male gametes produced by hexaploids were evidently viable and could effect fertilization. Whether this mechanism is also true for egg cells or whether the hexaploids are capable of producing unreduced embryo sacs is uncertain. Because of severe reproductive difficulties, hexaploid seedlings may play a very minor role in gene flow and the further evolution of *H. perforatum*. The likelihood that hexaploids will evolve to types with an increased frequency of bivalent paring in meiosis is relatively low. However, hexaploids may include novel chemotypes, which could be vegetatively propagated if valuable, medicinal types can be identified among them.

1. Introduction

Hypericum perforatum, commonly known as St John's wort, has long been used as a medicinal plant (Hobbs, 1989), recognized for its antiviral, anti-inflammatory, anti-depressive, and anticancer activities (Birt et al., 2009; Gartner et al., 2005; Kim et al., 1999; Kubin et al., 2005; Zanoli, 2004). Along with growing research interest in the medicinal properties of *H. perforatum* and its cultivation as a crop, there is also a growing body of reports on genetic investigations (Barcaccia et al., 2006; Mayo and Langridge, 2003; Matzk et al., 2001; Percifield et al., 2007), and germplasm collection and cultivar development (Bruni and Sacchetti, 2009; Franke et al., 1999; Gaudin et al., 2002; Mayo and Langridge, 2003).

Robson (1977) noted that *H. perforatum* was originally native to southern Europe, but is now commonly found throughout the temperate regions of both the Northern and Southern Hemispheres. Populations may include diploid, tetraploid, and hexaploid cytotypes (Noack, 1939; Robson and Adams, 1968; Robson, 2002), with tetraploids by far the most common and likely the ancestral type. Campbell and Delfosse (1984) suggested that *H. perforatum* was created via interspecific hybridization between two diploids ($2n = 16$): *H. maculatum* Crantz subsp. *maculatum* and *H. attenuatum* Choisy. Initial hybridization would need to have been followed by subsequent chromosome doubling, likely via unreduced-gamete production (Harlan and deWet, 1975). It also might be possible that the tetraploid was formed through the unification of two 2*n* gametes from the parental species. Tetraploid *H. perforatum* is capable of asexual reproduction by apomixis (Mártonfi et al., 1996; Noack, 1939), a reproductive pathway in which embryo development proceeds without fertilization or sexual recombination (Nygren, 1967). However, for most apomictic species, including *H. perforatum*, the endosperm often still requires fertilization for normal seed development. Many populations of *H. perforatum* are facultative apomicts, since sexual and aposporic reproduction can occur in the same plant (Matzk et al., 2001). In most reported cases of apomictic reproduction in *H. perforatum*, the embryo develops from an aposporic, unreduced egg cell (Koperdáková et al., 2004; Matzk et al., 2001, 2003). Based on results of the Flow Cytometric

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doi:10.1016/j.indcrop.2010.01.005

Seed Screen (FCSS), it is likely that diploid and hexaploid cytotypes are typically produced by tetraploid plants via haploid parthenogenesis and fertilization of unreduced egg cells, respectively (Matzk et al., 2001).

Apomictic reproduction in tetraploid *H. perforatum* first was described by Noack (1939) by examining cytotypes of offspring resulting from controlled crosses. Matzk et al. (2001) analyzed seed samples collected from 113 different populations of *H. perforatum*, providing a detailed characterization of apomictic reproductive pathways. They used FCSS to determine ratios of cellular nuclear DNA contents in individual-seed embryo tissue to those of the endosperm. For example, a tetraploid seed from a tetraploid mother plant generates two signal peaks: a 4Cx peak [Cx-value: DNA content of a monoploid genome with chromosome base number x (Greilhuber et al., 2005). Cx is used throughout the text to indicate DNA content detected by flow cytometry] and a 10Cx peak, which show that the embryo sac was unreduced and the egg cell developed parthenogenetically, while the center cell was fertilized by a reduced male gamete. In diploid seeds, a 2Cx peak and a 6Cx peak indicate that the embryo sac was reduced and the egg cell developed parthenogenetically, while the center cell was fertilized by a reduced male gamete. And in hexaploid seeds, a 6Cx peak and a 10Cx peak indicate that the embryo sac was unreduced, and both the egg cell and the center cell were fertilized by reduced gametes.

Since the pioneering work of Matzk et al. (2001), reproductive pathways in many more populations of *H. perforatum* and in other *Hypericum* species have been analyzed with FCSS (reviewed by Barcaccia et al., 2007). However, many of these studies evaluated bulked-seed samples, with individual seeds used only occasionally to provide supplementary data (Barcaccia et al., 2006; Koperdáková et al., 2004; Matzk et al., 2001). A wide range of reproductive pathways has been found in the genus, with eleven different routes to seed production described in *H. perforatum* after examining 113 accessions (Matzk et al., 2001). Both diploid and hexaploid seeds often have been found in these investigations. About 2% of the evaluated *H. perforatum* plants were shown to be diploid in a FCSS screen of 113 accessions of *H. perforatum*, and a single tetraploid plant was observed that could produce up to 75% hexaploid seed (Matzk et al., 2001).

Since apomictic reproduction in tetraploid *H. perforatum* imposes barriers to gene flow and recombination and, thus, to the generation of new genotypes, other cytotypes might help overcome this barrier. Diploid *H. perforatum* was described as being morphologically similar to the tetraploid type (Matzk et al., 2001), and diploids of many taxa can reproduce sexually. However, due to the mode by which these diploids are thought to be generated (non-recurrent apomixis), they may have limited fertility. No morphological or biochemical characterization of hexaploid *H. perforatum* plants has been reported, and their reproductive behavior is unclear. Matzk et al. (2001) used the observation of a 15Cx endosperm peak and a 6Cx embryo peak in an FCSS profile of a bulked-seed sample from a *H. perforatum* accession to infer that hexaploid plants could occur in that parental population, with seeds generated apomictically from unreduced hexaploid embryo sacs, but no further elaboration was given. To our knowledge, it is unclear whether any well-established *H. perforatum* populations with predominantly diploid or hexaploid cytotypes have been found in nature.

The presence of plants of different ploidy levels within a natural population could also affect the evolutionary course of that population, if the different cytotypes reproduce differentially or can cross among themselves with few significant barriers. In the case of *H. perforatum*, effective reproduction within each cytotype would probably lead to the evolution of separate diploid or hexaploid populations through drift or other stochastic processes. Normal sexual hybridization between different cytotypes would be expected to produce seeds with odd ploidy levels, which would likely be infertile or only reproduce via seed through some form of apomixis or union of unreduced gametes. The impact of hybridization among different cytotypes on populations will be greatest when the population size is small. This may be an important consideration in generating distinctly new populations in nature, in breeding and selection programs, and in *ex situ* conservation.

With regard to *ex situ* conservation, genebanks typically need to regenerate accessions when the overall quantity of seed is low or its viability declines (Sackville Hamilton and Chorlton, 1997). Plant populations used for seed regeneration are often relatively small, and may sometimes be less than 30, especially when a curator assumes that there is minimal within-population variation, as expected with apomicts. For *H. perforatum*, apomictic tetraploid plants should predominate in most populations as has been reported. However, in small populations, a few diploid and or hexaploid plants (if they produce normal viable gametes) could cause the regenerated seed population to contain a significant proportion of triploid, pentaploid or septaploid seeds, in addition to those of their own cytotype. This situation would have significant negative effects on seed quality and could worsen after successive cycles of serial regeneration.

Despite the potential negative impacts of the mixed-cytotype situation noted above, diploid and hexaploid *H. perforatum* plants could provide novel traits for crop development, such as disease resistance or phytochemical profiles with increased medicinal value. For example, Čellárová et al. (1997) reported that diploid somaclones of *H. perforatum* produced more hypericin, a pharmacologically important naphthodianthrone unique to Clusiaceae (Hölzl and Petersen, 2003), than did triploids or tetraploids. Our investigation was initiated to study cytotype composition and reproductive pathways of three *H. perforatum* accessions maintained by the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa and evaluate the potential impacts on seed reproduction by diploids and hexaploids, if present.

2. Material and methods

2.1. Plant material

Seed and leaf samples were analyzed in this experiment. Seeds of three randomly chosen *H. perforatum* accessions were sampled from the NCRPIS, two accessions (Ames 27490 and PI 325351) originally collected in Russia and one accession (Ames 28292) originally collected in California. Seed lots were regenerated in isolated cages at the NCRPIS and were one generation removed from the original collections. One hundred individual seeds were sampled from each lot. To obtain leaf samples, seeds from these same lots were placed on water-saturated blotter paper in clear, plastic germination boxes held in a germination chamber at 30/20 °C (16/8 h) under continuous fluorescent light. Seedlings were transplanted into flats and grown in the NCRPIS greenhouse before field planting. Individual leaf samples from 30 plants of Ames 27490 and Ames 28292 were collected from greenhouse-grown plants, and 35 plants of PI 325351 were individually sampled from field transplants.

2.2. Flow cytometry

Single-seed nuclei suspensions were created by placing individual seeds in a tube containing 1 mL nuclei-stabilizing buffer (15 mM HEPES, 1 mM EDTA, 80 mM KCl, 20 mM NaCl, 300 mM Sucrose, 0.2% Triton X-100, 0.5 mM Spermine tetrahydrochloride, 0.25 mM PVP) and disrupting them with a homogenizer (Omni International 1000; Waterbury, CT) for about 8 s at 15,000 rpm. The homogenized suspension was supplemented with an additional 1 mL of nuclei-

Table 1
Number and types of seeds and plants of three *Hypericum perforatum* accessions by flow-cytometry analysis.

Accession	No. of seeds with embryo/endosperm ratio and of plants with ploidy levels													Total	%		
	2Cx/4Cx	(2Cx + 4Cx)/6Cx	2Cx/6Cx	2Cx/10Cx	4Cx	4Cx/4Cx	4Cx/6Cx	4Cx/8Cx	4Cx/9Cx	4Cx/10Cx	4Cx/13Cx	6Cx	6Cx/10Cx	6Cx/13Cx		2Cx	4Cx	6Cx
PI 325351																		
Seed						2	6	1		75	1		14	1	100		86	14
Plant					32							3			35		91	9
Ames 28292																		
Seed						2	8	1	1	78	1		8	1	100		91	9
Plant					26							4			30		87	13
Ames 27490																		
Seed	1	1	2	1		3	34	2	2	40	4		7	2	100	5	85	9
Plant					29							1			30		97	3

stabilizing buffer, passed through a 20 μm nylon mesh filter (Small Parts Inc.; Miramar, FL), and then centrifuged for 6 min at 100 × *g*. After discarding the supernatant, the nuclei pellet was resuspended with 250 μL staining buffer (10 mM $MgSO_4$, 50 mM KCl, 5 mM HEPES, 0.1% DL-dithiothreitol, 2.5% Triton X-100, 100 μg/mL propidium iodide) and then analyzed by flow cytometry for relative nuclei DNA content. All flow cytometry was performed with a BD Biosciences (San Jose, CA) FACSCanto, equipped with a 488 nm laser and 610/20 emission filter.

Nuclei suspensions also were created from mature leaf samples. About 0.5 g of leaf tissue was collected from fully or near fully expended leaves of individual seedlings, taken 2 months after germination. The tissue was placed in a 60 mm × 15 mm polystyrene dish with 2.5 mL nuclei-stabilizing buffer, chopped to a fine mash with a single-edge razor blade, and then filtered and stained as above.

Reproductive pathways were determined by comparing the ploidy levels of the embryo tissue with that of the endosperm tissue in a seed. Mature seeds of *H. perforatum* include cells representing both the embryo and endosperm that still retain intact nuclei, but biased towards the embryo. This results in relatively small peaks for the endosperm and larger ones for the embryo in FCSS histograms (Matzk et al., 2001). Interexperiment cytometer performance was tracked by using the BD Bioscience CST bead system, and the linearity of DNA measurement was calibrated via the standard chicken-erythrocyte nuclei method (Vindelov et al., 1983).

3. Results

Nuclear DNA contents of the embryo and endosperm tissue of seeds can be differentiated through their peak locations on flow-cytometry histograms (Matzk et al., 2000, 2001). In contrast, preliminary experimental tests showed that seed size was not a reliable indicator of ploidy for these accessions (data not shown). All three populations produced seeds with different cytotypes and embryo–endosperm ratios (Table 1, Fig. 1). No seeds with triploid, pentaploid, or heptaploid embryos were found in these populations. Embryos with DNA content corresponding to the tetraploid level were the predominant cytotype (85–91%) in all populations, with a smaller proportion of hexaploid embryos (9–14%), and diploid embryos (5%) detected only in Ames 27490. A single seed containing a twin embryo of 2Cx and 4Cx was also found in the Ames 27490 population. On the endosperm side, the predominant type was decaploid, generally associated with the tetraploid and hexaploid embryos. However, endosperm tissue with other ploidy levels ranging from 4Cx to 13Cx was also detected at low frequencies (Table 1).

In the seedling populations, only tetraploids and hexaploids were found (Table 1), with tetraploids constituting 87–97% of the populations. No obvious morphological differences were observed between the tetraploid and hexaploid seedlings.

4. Discussion

The seed samples tested included a high proportion of tetraploid embryos along with fewer diploids and hexaploids, consistent with past descriptions of other *H. perforatum* populations (Barcaccia et al., 2006; Matzk et al., 2001). The presence of hexaploid plants at low frequencies in all three seedling populations suggests that hexaploid plants were present within the parental populations from which the seeds were originally collected. However, resampling the original populations is impossible, and sufficient quantities of the original seeds are unavailable for two of these accessions.

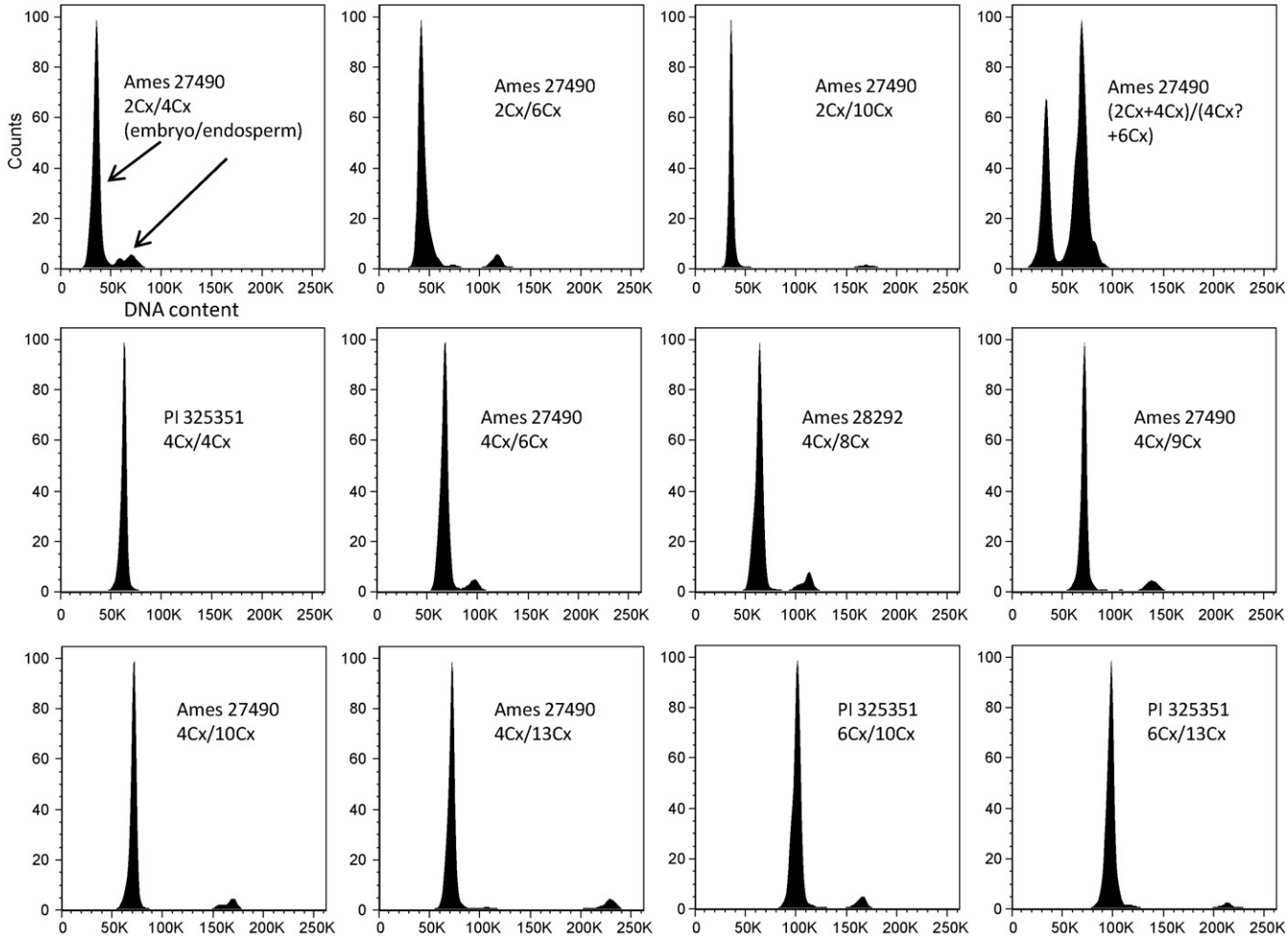

Fig. 1. Typical flow-cytometry histograms of single-seed samples of three *Hypericum perforatum* accessions.

From the various ratios of embryo and endosperm DNA content, it is clear there were several reproductive pathways leading to seed production. All three accessions were facultatively apomictic, since they all produced some seeds with sexually generated embryos. "Normal," reduced embryo-sac formation was evidenced in all three populations, and most of these embryo sacs developed into 4Cx:6Cx seeds (Table 1), via double fertilization by reduced male gametes from the tetraploid. However, the common association of decaploid endosperm with tetraploid or hexaploid embryos (Table 1) indicates that, most often, unreduced embryo sacs developed in tetraploid plants and that some of those embryo sacs were fertilized by reduced (diploid) male gametes from the tetraploids. Double fertilization resulted in the 6Cx:10Cx seeds, and single fertilization of the central cell (pseudogamy) produced the 4Cx:10Cx seeds, these being the two most common types observed (Table 1). 4Cx:4Cx seeds (Table 1) could develop from a reduced embryo sac following fertilization of the egg cell by a reduced male gamete, with "automatic" development of the central cell into endosperm, and 4Cx:8Cx seeds could result from the automatic development of an unreduced embryo sac. Seeds with 2Cx embryos were found only in Ames 27490 (Table 1) and apparently were generated by parthenogenesis of reduced egg cells from a tetraploid parent, based on the associated 4Cx, 6Cx, and 10Cx endosperm types. At present, it is unclear what mechanism was responsible for the production of a single twin-embryo seed (2Cx + 4Cx):(4Cx + 6Cx). Twin-embryo seeds previously have been reported in *H. perforatum* (Matzk et al., 2001) and in other species, including *Pelargonium* × *hortorum* L.H. Bailey (Kubba and Tilney-Bassett, 1980) and *Prunus dulcis* (Mill.) D.A. Webb (Martínez-Gómez et al., 2003).

The presence of mature seeds of normal appearance with diverse embryo and endosperm ratios suggests that *H. perforatum* can tolerate deviations from the critical ratio of maternal (m) and paternal (p) genomes in the endosperm, which in most angiosperms is 2m:1p (Spielman et al., 2003). Endosperm with a 4m:1p ratio, a common product of interploidy crosses in gametophytic apomixis involving the production of unreduced embryo sacs, is often lethal (Haig and Westoby, 1991). However, since this ratio is dominant in these seed populations, it apparently has no negative effect on seed production or its viability in *H. perforatum*. This endosperm ratio also has been reported to be fully functional in *Tripsacum dactyloides* (L.) L. (Brown and Emery, 1958), with the same formation mechanism as reported for *H. perforatum*. As FCSS is destructive, it is not known whether non-4m:1p ratios influence *H. perforatum* seed viability, but the 2Cx:4Cx, 2Cx:6Cx, and 2Cx:10Cx seeds which were present at a frequency of 5% in Ames 27490, did not correspond to any diploid seedlings among the 30 sampled.

The fact that we found no triploid, pentaploid, or heptaploid seeds is also very informative regarding reproductive pathways in these *Hypericum* accessions. First, it suggests that a fertile diploid was probably absent or very rare in the parental populations; otherwise, seeds with diploid, triploid and pentaploid embryos should be detected. Second, it provides evidence that hexaploid plants, if present in the parental population, rarely, if ever, produce bal-

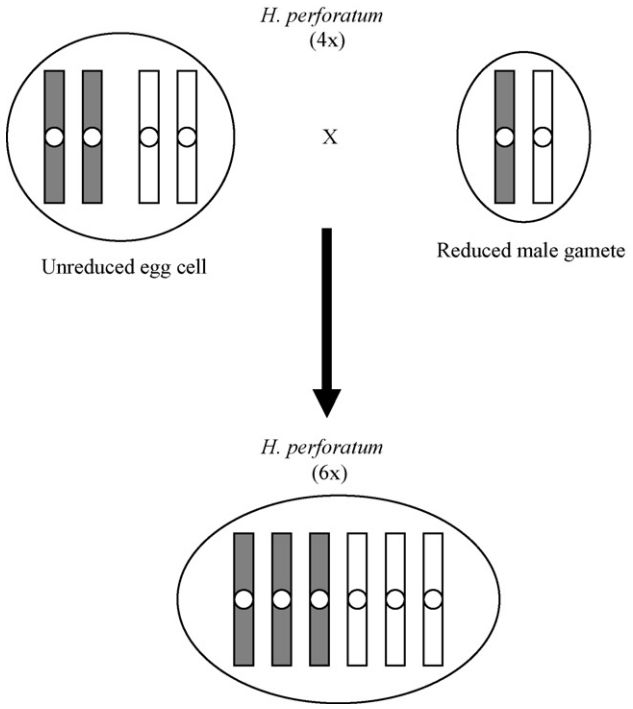

Fig. 2. An illustration of the generation of hexaploid *H. perforatum* from tetraploid *H. perforatum* and its genetic composition, with chromosome shading added to reflect the two ancestral species.

anced reduced gametes, because that could lead to pentaploid and heptaploid seeds.

The presence of some seeds with unusual embryo:endosperm ratios, such as 4Cx:9Cx, 4Cx:13Cx, and 6Cx:13Cx (Table 1, Fig. 1), suggest that unbalanced male gametes, which were most likely generated by hexaploids, were involved in the production of these seeds. Tetraploid unreduced embryo sacs were most likely involved in the formation of 4Cx:13Cx seeds, since embryo sacs of this type were so common in all three populations. If that is the case, the 13Cx endosperm probably resulted from the union of the central cell with an unbalanced, pentaploid male gamete produced by a hexaploid plant. The 4Cx:9Cx seeds were noted in Ames 27490 and Ames 28292, which produced 42% reduced embryo sacs based on seeds with 2Cx and 4Cx embryos with ≤6Cx endosperm. Since diploid plants were absent (or at least contributed no seeds) to these populations, the 4Cx:9Cx seeds would likely be the product of an independent fertilization of a reduced tetraploid embryo sac by a reduced tetraploid male gamete to the egg cell and by an unbalanced pentaploid male gamete to the central cell. The 6Cx:13Cx seeds could be produced in a similar fashion, but from an unreduced tetraploid embryo sac.

These possibilities suggest that hexaploid plants derived from tetraploid plants display meiotic abnormalities, leading to unbalanced-gamete production and reproductive difficulties. The inability of hexaploids to produce balanced gametes is likely a direct consequence of their genetic origin. Based upon the hypothesis of an allopolyploid origin for tetraploid *H. perforatum* (Campbell and Delfosse, 1984), a diagram of the genetic makeup of a hexaploid derived from tetraploid parents is presented in Fig. 2. Alternatively, an autopolyploid origin of tetraploid *H. perforatum* recently was proposed by Barcaccia et al. (2007) based upon a cytogenetic study of fluorescence-labeled karyotypes (Brutovská et al., 2000) and on observations that some diploid *H. perforatum* plants could produce viable seeds (Barcaccia et al., 2007). Because tetraploid *H. perforatum* plants often produce regular, reduced male gametes, a degree of diploidization and chromosomal differentiation must have been achieved during the course of its evolution. Thus, the cytological behavior of the genome of a hexaploid *H. perforatum* originating from diploidized, tetraploid parents would resemble that described in Fig. 2, except that differentiation between chromosome pairs may be less distinct.

It is reasonable to believe that such hexaploids would display abnormal meiotic behaviors, such as lagging chromosomes and unequal separation at cytokinesis (Stebbins, 1947). Many unbalanced gametes would likely abort before anthesis or fertilization, but some male gametes retaining more than half of the genome theoretically could survive, such as the hypothetical pentaploid gametes noted earlier. We have no evidence that this is true for egg cells based upon our FCSS results, although Park et al. (2002) noted that, in triploid grapes (*Vitis* spp.), female gametes are sometimes more tolerant to unbalanced chromosome sets than are male gametes.

In plants, unreduced-gamete formation, complement fractionation (Thompson, 1962) and cytomixis (Tyagi, 2003) are mechanisms that overcome reproductive obstacles caused by reduced meiotic chromosome paring. Matzk et al. (2001) suggested that formation of unreduced embryo sacs and normal reduced microsporogenesis might be possible in some hexaploid *H. perforatum* plants, based upon the observation of a 15Cx peak in the FCSS of bulked *H. perforatum* seeds. We agree that it is reasonable to hypothesize that hexaploids can form unreduced embryo sacs given its high frequency in tetraploids. However, it may be tenuous to predict the presence of normal microsporogenesis based upon the observation of a 15Cx peak in a bulked-seed sample. As we discussed, the presence of normal reduced microsporogenesis in hexaploid *H. perforatum* is unlikely. As an alternative hypothesis, such seeds might be produced by parthenogenesis of an unreduced or an unbalanced hexaploid embryo sac, with cellular DNA content close to 6Cx, fertilized by an unbalanced male gamete from the hexaploid, with DNA content close to 3Cx. Furthermore, multiple fertilization of a central cell is another potential route for higher ploidy levels in the endosperm of *H. perforatum*. This mechanism has been observed in *Arabidopsis thaliana* (L.) Heynh. (Spielman et al., 2003). Our observation of 2Cx:10Cx seeds (Table 1) may offer support for this mechanism.

If hexaploid *H. perforatum* plants can produce unreduced embryo sacs and approximately normal, triploid male gametes, one might expect that seeds with higher ploidy embryos than hexaploid, such as septaploid, octoploid or nonaploid, could be found via FCSS in mixed populations. However, these embryo types were observed neither in previous FCSS studies (reviewed by Barcaccia et al., 2007) nor in our investigation. It is perhaps rather unlikely, but still possible, that hexaploids could produce a range of unbalanced male and female gametes with relatively complete chromosome complements through processes analogous to complement fractionation in *Rubus* (Thompson, 1962) or cytomixis in *Mentha* (Tyagi, 2003), leading to seeds with unusual embryo:endosperm ratios. We are now investigating this topic in more detail by following pollen and seed production in hexaploid seedlings.

In crosses between plants from two different *H. perforatum* populations which were highly apomictic, Mayo and Langridge (2003) found that only 2% (3 out of 113) of the progeny were hexaploid. Our tested populations contained 3–13% hexaploid seedlings. To our knowledge, no established hexaploid *H. perforatum* populations have been found in natural habitats, even with the finding that a tetraploid *H. perforatum* plant can produce up to 75% hexaploid seeds (Matzk et al., 2001). If plants resulting from hexaploid seeds can reproduce through either normal sexual or apomictic means, it should be possible to establish hexaploid populations in nature. With two sets of autotriploid chromosomes, it seems difficult for a hexaploid *H. perforatum* to evolve towards a more normal meio-

sis. In the tetraploids, their putative allopolyploid origin and the production of normal, reduced male gametes indicate that bivalent paring can be achieved during meiosis. Therefore, for hexaploids to do the same, the chromosomes introduced into the tetraploid genome by the reduced male gametes must also form bivalents. However, since the newly introduced chromosomes were two sets originally from the same species that generated the tetraploid, it would be highly unlikely for them to pair uniquely with each other in the hexaploid cell environment initially or to evolve such bivalent pairing. The fate of hexaploid plants in natural populations is unknown. However, their accumulation in *H. perforatum* populations over time seems quite unlikely, and we expect their contribution to gene flow or the species' further evolution to be minimal.

Our investigation provides additional support to previous findings that apomictic reproduction through unreduced embryo-sac production is very common in tetraploid *H. perforatum* and that hexaploid seeds are produced when unreduced embryo sacs are double fertilized by reduced tetraploid pollen. Because of their expected infertility, hexaploids should have very limited impact on seed regeneration, both *in situ* and for *ex situ* conservation. Any potential utilization of hexaploids is currently unclear. While they may have little direct value in breeding, it would be useful to evaluate the phytochemical profiles of hexaploids for medicinal constituents. If hexaploid plants with high levels or novel combinations of bioactive chemicals can be found, vegetative propagation of such plants through *in vitro* or traditional methods could be exploited, and the generation of larger numbers of hexaploids through controlled crosses among parental types of *H. perforatum* with superior agronomic characteristics warranted.

Acknowledgments

The authors wish to thank Drs. Reid G. Palmer, James F. Hancock and Thomas Ranney for useful critiques of this manuscript. This journal paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 1018, was supported by Hatch Act and State of Iowa funds, and the research described herein was supported by Award Number P50AT004155 from the National Center for Complementary & Alternative Medicine. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Complementary & Alternative Medicine or the National Institutes of Health. Mention of commercial brand names does not constitute an endorsement of any product by the U.S. Department of Agriculture or cooperating agencies.

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